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ENGINEERING SOLUTIONS FOR ROBUST AND EFFICIENT MICROFLUIDIC BIOMOLECULAR SYSTEMS: MIXING, FABRICATION, DIAGNOSTICS, MODELING, ANTI-FOULING AND FUNCTIONAL MATERIALS



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This technical report has been reviewed and is approved for publication.

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14. ABSTRACT

We have developed several key components for microfluidic bioanalysis systems, including the following:

- 1. Microfluidic component fabrication and prototyping
- Development of microflow diagnostic tools
- Micron-scale fluid flow mixing enhancement
- Flow modeling of micromixing processes
- Biomolecular fluid stream handling and anti-fouling techniques
- Spatial and temporal zeta potential control
- Integration of functional materials in microfluidic systems.

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Final Report

Engineering Solutions for Robust and Efficient Microfluidic Biomolecular Systems: Mixing, Fabrication, Diagnostics, Modeling, Anti-fouling and Functional Materials

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Project Summary

On this project we produced engineering solutions in five key areas of biomolecular Microfluidic Component Fabrication and Prototyping, microfluidic system design: Development of Microflow Diagnostic Tools, Micron-Scale Fluid Flow Mixing Enhancement, Flow Modeling of Micromixing Processes, and Biomolecular Fluid Stream These technologies are fundamental to the Handling and Anti-Fouling Techniques. development of efficient, multi-step, multi-use microfluidic bioanalysis systems. developments in microfluidic prototyping and flow field diagnostic tools have empowered experimental studies of biomolecular microfluidic systems. The micron-scale fluid mixing enhancements we have developed - most notably applying the science of chaotic advection to enhance mixing in the microscale - has had a significant impact on the field. Solutions in all the five key areas have been developed for pressure driven and electrokinetic driven flow regimes. Further, the integration of functional materials with micro fluidic systems was investigated leading to the demonstration of spatially and temporally controlled zeta potentials in micro channels and initial demonstrations of autonomous components.

We have developed several key components for microfluidic bioanalysis systems including:

- 1. Microfluidic Component Fabrication and Prototyping
- 2. Development of Microflow Diagnostic Tools
- 3. Micron-Scale Fluid Flow Mixing Enhancement
- 4. Flow Modeling of Micromixing Processes
- 5. Biomolecular Fluid Stream Handling and Anti-Fouling Techniques
- 6. Spatial and Temporal Zeta Potential Control
- 7. Integration of Functional Materials in Micro Fluidic Systems

An understanding of these basic processes (in both pressure and electrically driven flow regimes) is crucial to the development of general design methodologies for scaled-down, complex, re-usable bioanalysis systems. The developments in microfluidic prototyping and flow field diagnostic tools will serve microfluidic design efforts in general. The micron-scale fluid mixing enhancements and modeling we propose - most notably the science of chaotic advection to enhance mixing at the microscale - presents a transforming technology the can be used to optimize a wide range of bioanalysis processes and provide robust, easily controlled passive micromixers. The design methodologies and cleaning procedures for anti-fouling issues in bioanalysis systems is an important and necessary step to the design of multi-step, re-usable systems. Spatial and temporal control of zeta potential will provide for chaotic mixing in electrokinetic flows, while the integration of functional materials into micro fluidic systems may open up entirely new fabrication and device modalities. The specific, innovative methods with that we have developed in each of these areas is summarized below and in the attached publications. The project was successful because of the breadth of expertise brought to bear. The project managed to establish a uniquely productive dialog between theoreticians, experimenters and manufacturers. It spanned a surprising array of departments and topical expertise, from chemistry and chemical engineering to electrical engineering to mechanics, physics and applied mathematics. The computational diagnostic toolkit that we introduced in the modeling nicely complemented the experimental techniques (both micro-PIV and flow The notion that geometry could be quickly varied, and that different visualization). geometries thus could be explored, was made possible by the dialog between modeling and manufacturing. One could say that we achieved a level of 'rapid prototyping' through this dialog. Turnaround times for an insight developed through the modeling and the availability of a manufactured device for laboratory trials was really quite short, often on the order of days. The major accomplishments of the project are briefly summarized below. Complete details are available in the attached publications.

Microfluidic Component Fabrication and Prototyping

In order to achieve passive chaotic advective mixing spatial control in three dimensions is required. This provided the motivation for the development of the first rapid prototyping methods for three dimensional microfluidic channel networks. Early MEMS developments were largely based on conventional semiconductor materials and techniques originally developed for the integrated circuit industry. Use of these materials and techniques for developing micro-fluidic devices has not only resulted in high cost but also many limitations on fabrication, packaging and testing. Another polymer, PDMS, is receiving an increasing amount of attention from the micro-fluidics field. PDMS micro-molding techniques have been used to fabricate micro-fluidic systems by several groups. Unlike traditional micro fabrication materials such as silicon and glass, PDMS is a low cost material. Micro molding processes are simple and rapid when compared to traditional etching and bonding approaches. The primary advantages of PDMS material for micro-fluidics applications include ease of bonding, optical properties (transparent from 230 to 700 nm wavelength within the range of 190 to 700 nm), and permeability to gases for some biological applications. Therefore, PDMS is particularly suitable for prototyping and testing various micro-fluidic devices. Although the scientific community has recently witnessed several 3D component designs, the majority of devices were fabricated with silicon and related materials using complicated and time consuming processes. Moreover, the wet etching of single crystal silicon is geometrically limited by the crystallographic planes. Expensive dry etching equipment is necessary to make curved vertical walls in a silicon wafer. Thus, fabricating 3D channel paths in silicon is often non-trivial and silicon is not transparent to most wavelengths of interest for biological applications.

We have developed a simple micro-fabrication technique that allows the rapid construction of complex 3D micro fluidic channels. A parametric study of PDMS bonding via plasma activation is also reported. The techniques described can be applied to realize various kinds of 3D micro-fluidic systems such as micro-mixers, micro-valves, capillary electrophoresis (CE) systems and micro total analysis systems (μTAS). The micro-fabrication technique developed for constructing complex 3D channel paths is based on the stacking of thin (~100 μm) two-dimensional (2D) patterned PDMS layers and is described in detail in attached publications.

Development of Microflow Diagnostic Tools

We have made key advances in the development of scalar and particle based diagnostics and the development of two key electrokinetic device components: Optimized electrokinetic turns and electrokinetic instability micromixers. Below is a summary of this work. Additionally we have developed advanced algorithms for analysis of Particle Velocimetry images for investigation of microscale flows. Furthermore these algorithms were applied to a variety of microchannel flows.

μPIV and PTV for Electrokinetics

We extended micron-resolution particle image velocimetry (μ PIV) to make fluid velocity measurements in electrokinetic flows with spatial and temporal resolution of 1 um and <10 ms, respectively. This methodical and quantitative extension of this powerful diagnostics technique included a method of measuring electrophoretic mobilities of large ensembles (> 3000) of seed particles using the μ PTV technique (micron-resolution particle tracking velocimetry); a technique we developed for this purpose. In this μ PTV quantification of mobility distributions, we tracked thousands of electrophoretic particles while simultaneously measuring electroosmotic wall mobilities using the current monitoring method. This particle tracking work was used to provide a quantitative confirmation of the similarity between the electric field and electroosmotic flow field in a microfluidic system. Our techniques were also applied in demonstrating simultaneous measurements of two distinct electroosmotic mobilities in a microfluidic channel with variable zeta potential. This work was published in two journal papers.

Full Field Visualizations with Line Writing

Full field imaging with two fluorescence-based line writing techniques was successfully applied and the work has been published in several archival journals. The first technique, bleached fluorescence visualization, is an important innovation that was implemented in performing simultaneous measurements of diffusivities and electrophoretic mobilities of fluorescent bands. The technique was demonstrated in both glass and acrylic microchannels. A caged fluorescence visualization setup, along with the bleached visualization technique, was built and applied to study the turn geometry optimization work discussed below.

Particle Image Velocimetry Measurements of a Microchannel Flow

A Particle Image Velocimetry (PIV) system has been developed to measure velocity fields with order one-micron spatial resolution. The technique uses 200 nm diameter flow-tracing particles, a pulsed Nd:YAG laser, an inverted epi-fluorescent microscope, and a cooled interline-transfer CCD camera to record high-resolution particle-image fields. The spatial resolution of the PIV technique is limited primarily by the diffraction-limited resolution of the recording optics. The accuracy of the PIV system was demonstrated by measuring the known flow field in a 30 $\mu m \times 300~\mu m$ (nominal dimension) microchannel. The resulting velocity fields have a spatial resolution, defined by the size of the first window of the interrogation spot and out of plane resolution of 13.6 $\mu m \times 0.9~\mu m \times 1.8~\mu m$, in the streamwise, wall-normal, and out of plane directions, respectively. By overlapping the interrogation spots by 50% to satisfy the Nyquist sampling criterion, a velocity-vector spacing of 450 nm in the wall-normal direction is achieved. These measurements are accurate to within 2% full-scale resolution, and are the highest spatially resolved PIV measurements published to date.

Central Difference Interrogation Algorithm

An adaptive, second-order accurate particle image velocimetry (PIV) technique has been developed. The technique uses two singly pulsed images that are interrogated using a modified cross correlation algorithm. At the heart of the algorithm is a central difference approximation to the flow velocity (accurate to order Δt^2) versus the forward difference approximation (accurate to order Δt) common in PIV. An adaptive interrogation region shifting algorithm is used to implement the central difference approximation. The adaptive central difference interrogation (CDI) algorithm has two main advantages over adaptive forward difference interrogation (FDI) algorithms: it is more accurate, especially at large time delays between camera exposures; and it provides a temporally symmetric view of the flow.

Ensemble-averaged Correlation Algorithm

A PIV algorithm is developed for estimating time-averaged or phase-averaged velocity fields. The algorithm can be applied to situations where signal strength is not sufficient for standard cross correlation techniques, such as a low number of particle images in an interrogation spot, or poor image quality. The algorithm can also be used to increase the spatial resolution of measurements by allowing smaller interrogation spots than those required for standard cross correlation techniques. The quality of the velocity measurements can be dramatically increased by averaging a series of instantaneous correlation functions, before determining the location of the signal peak, as opposed to the commonly used technique of estimating instantaneous velocity fields first and then averaging the velocity fields.

Electrokinetic Channel Turns with Minimal Dispersion

A major additional accomplishment of our project (not originally anticipated) was the minimization of dispersion of electrokinetically advected analyte bands in microchannel turns. Optimizing microchannel turn geometry, we achieved a 98% reduction in the dispersion variance in our geometries compared to the dispersion of a constant radius turn. The publication record shows that our group was the first to innovate these optimal geometries for electrokinetic (including electrophoretic) systems. Our optimization approach combined the development of analytical and computational modeling with an experimental validation of the

turn geometry. Both caged and photobleached fluorescence techniques were used to demonstrate dispersion reduction.

Biomolecular Fluid Stream Handling and Anti-Fouling Techniques

Nonfouling Coatings for Microfluidic Devices

To develop coating methodologies that effectively reduce nonspecific protein adsorption and channel fouling, we explored two different surface modification strategies. The first used the plasma deposition of ethylene oxide based coatings. This approach has been shown to effectively resist protein fouling in macroscopic systems. We explored several different deposition conditions, including RF power in the plasma reactor, the starting monomer, partial pressure of the monomer, carrier gas, etc. Channel coating and subsequent protein deposition studies demonstrated that these coatings are all similarly successful as lining materials for polydimethylsiloxane-based microfluidic devices. Because the ethylene oxide coatings are not particularly chemically reactive, they are less amenable to chemical grafting of enzymes and other sensing proteins. We therefore developed a second approach to preventing biofouling based on the covalent attachment of blocking proteins to the PDMS walls. The proteins used have been shown to successfully prevent protein adsorption in a number of antibody-based assays (see below).

Immobilized Enzyme Technology

Microfluidic Based on chip ELISA Assay.

An "on chip" enzyme linked immunoassay (ELISA) sensor was successfully developed using a PDMS based microfluidic device. This standard immuno-detection assay was miniaturized and shown to be as sensitive as the standard 96-well plate format used in immunodiagnostics. Miniaturization offers the advantage of higher throughput in a shorter time period. The use of immobilized albumin for preventing nonspecific adsorption in the device, as discussed above, increased the sensor detection signal to noise substantially above that achieved with uncoated PDMS channels.

Heterogeneity

A quantitative analysis method was developed for the quantification of immobilized protein heterogeneity in immobilized protein-based biosensors. Heterogeneity that often accompanies protein immobilization is a significant limitation to biosensor performance. Using the Sips isotherm to analyze analyte binding to immobilized antibodies, we quantified the protein heterogeneity in each preparation. This approach was used to identify immobilization conditions that minimize immobilize protein heterogeneity, and we demonstrated that this results in more uniform, reproducible protein preparations and higher quality sensor data.

Immobilization Strategies

Strategies for controlling the orientation of immobilized proteins were developed using genetic engineering approaches. The strategic positioning of reactive handles on protein surfaces resulted in uniformly oriented, immobilized proteins, which exhibited homogeneous properties, and improved the activity of biological sensors in which these tools were employed.

Micron-Scale Fluid Flow Mixing Enhancement & Flow Modeling of Micromixing Processes

The μ Flumes project may be remembered as the breakthrough point for the idea of chaotic advection in microfluidics. Although Liepmann & Pisano had explored the idea of using chaotic advection in a pulsed micro-mixer previously, and although the functioning of empirically constructed micro-mixers in many cases hinges on chaotic advection but was not understood in those terms, this was the first project in which the idea of 'designing for chaos' motivated the work from start to finish and in which consistently functioning and well characterized devices were produced. Clear comparative tests between various geometries, backed up by numerical simulations that established the presence or absence of chaotic advection, were performed under μ Flumes, and the results were reported in formats acceptable to both the microfluidics community and the established fluid mechanics community.

Rapid mixing is essential in many of the microfluidic systems targeted for use in biochemistry analysis, drug delivery, and sequencing or synthesis of nucleic acids, among others. Biological processes such as cell activation, enzyme reactions, and protein folding often involve reactions that require mixing of reactants for initiation. Mixing is also necessary in many microfabricated chemical systems that carry out complex chemical synthesis.

When the dimensions of a channel cross-section are tens of micrometers, molecular diffusion can mix two fluid streams in just a few seconds. However, when the dimensions are several hundred micrometers, a molecular diffusion-based mixing process can take tens of seconds. Mixing is particularly inefficient in solutions containing macromolecules that have diffusion coefficients one or two orders of magnitude lower than that of most liquids. Effective mixing at this scale requires that fluids be manipulated to increase the interfacial surface area between initially distinct fluid regions so that diffusion can complete the mixing process in a reasonable time. Unfortunately, the rapid mixing produced by turbulent flows is usually not available at the microscale because the Reynolds number, Re, is typically below the critical value for transition to turbulence. Thus, some other mechanism must be used to enhance mixing. While the literature contains a number of devices designed to enhance mixing on the microscale, the focus here is thus on passive mixing because it is, in general, relatively simple to implement. It has been shown that a 'twisted pipe' has the potential to enhance mixing even at low Reynolds numbers. This mixing enhancement is possible because of the phenomenon known as chaotic advection, in which simple, regular velocity fields produce chaotic particle trajectories. Dynamical systems theory shows that chaotic particle motion can occur when a velocity field is either two-dimensional and time-dependent or threedimensional (with or without time dependence). The occurrence of chaotic advection typically indicates rapid distortion and elongation of material interfaces. This process significantly increases the area across which diffusion occurs, which leads to rapid mixing. On the macroscale, 'twisted-pipe' configurations have proven to be very effective mixers for We have developed a microchannel design based on the 'twisted pipe' and documented the mixing performance of the flow in this channel for Re from 6 to 70. In order to mix well at low Reynolds numbers, the geometry of a channel must be 'complicated enough' that chaotic advection results. However, in order for the channel to be easily fabricated and integrated into existing microfluidic systems, the geometry should remain relatively simple. It is the optimization of these competing factors that drove our design.

Flow visualization experiments confirm that the three-dimensional serpentine channel mixes significantly better than the square-wave channel and a straight channel for Reynolds numbers from 6 to 70. Mixing in the serpentine channel relies on the flow field being sufficiently three-dimensional, with secondary flows stretching and folding the fluid, greatly increasing the interfacial area across which diffusion occurs. The experimental results suggest the occurrence of chaotic advection in the serpentine micromixer. At a Reynolds number of 70 the serpentine channel produces 16 times more reacted phenolphthalein than a straight channel and 1.6 times more than the square-wave channel. The passive three-dimensional serpentine micromixer has a number of advantages that makes it attractive for use in a wide variety of microfluidic applications. First, this design is easy to fabricate and integrate with other microfluidic components. The results shown here are for essentially steady flow, but using this mixer in an application with time-dependent flow should enhance, not degrade, its performance. There is also some flexibility in the choice of channel geometry, as the occurrence of chaotic advection is not specific to the serpentine channel presented here. We are currently examining other channel designs. Secondly, the occurrence of chaotic advection depends on the global three-dimensionality of the flow field, not on high local rates-of-strain. Thus, using chaotic advection to mix biomolecular streams may help minimize damage to large biomolecules, some of which are particularly prone to shear-induced damage. Finally, keeping the flow in a single channel with a height-to-width ratio near one maintains a relatively low surface-to-volume ratio, which minimizes the chances of clogging, fouling, and loss of sample by biomolecular adsorption onto the device surface.

The main accomplishments of the modeling were both to suggest geometries to be explored and to provide supportive computations to round out the physical picture where direct experimental diagnostics became difficult or impossible (e.g., the construction of a Poincaré section). The size of the channels was such that a continuum approach was fully adequate and the usual ideas of Newtonian fluid mechanics were sufficient as a guide to the experiments. In this sense, the project did not reveal any physics that was not already understood. However, pushing these ideas to the level of tubes of 100-micron diameters, and seeing them work so well, was extremely satisfying and certainly not obvious a priori.

Chaotic Advection Mixing and Biosensor Performance

The efficacy of micromixers based on novel channel architectures was demonstrated with a protein-based biosensor. This study tested the ability of improved mixing to decrease the response time of an immobilized protein-based, microfluidic sensor platform. Chaotic advection in serpentine channels reduced the sensor response time at least two-fold under the limited conditions examined. The full range of experimental parameters is still being explored, but these promising results suggest that much greater rate enhancements should be accessible with these mixing configurations.

Electrokinetic Instability Micromixer

Our work in the area of particle tracking led us to a new and unexpected innovation of electrokinetic instability micromixers. These micromixers achieve rapid mixing using a flow instability that we discovered as part of this project. Rapid mixing is achieved in low (< 0.1) Reynolds number flows typical of microfluidic systems. This research encompasses the study of the physics behind this instability along with the optimization of the mixer given system

characteristics (e.g., buffer chemistry, substrate material, and geometry). We have continued this work under a new DARPA grant (see section on funding resulting from this work).

Spatial and Temporal Zeta Potential Control

The motivation to build a lab-on-a-chip for quick analysis has resulted in the fabrication of multi-function devices. The bulk flow velocity in microchannels can be manipulated by changing the direction and magnitude of the applied electric field. However, modulation of the flow without changing the potential difference would be beneficial in terms of separation efficiency. To improve the efficiency of separation in CE, some of the techniques studied are application of radial fields and surface coatings on the channel walls. To control flow direction, manipulation of surface pressure using electrochemical principles has been demonstrated. However, the resolution is limited in these techniques due to fringing effects and difficulties in controlling the fluid dynamics in a spatially restricted region. modulation via light may simplify device fabrication by reducing the electrical complexity (i.e. reduced lead count) and operation by allowing precise spatial and temporal control. In this paper, the active control of the bulk electroosmotic flow (EOF) velocity with light is demonstrated. Direct semiconductor like TiO2 exhibits a change in the surface charge upon irradiation with UV light. Earlier reports have shown that the electrophoretic mobility of TiO2 particles is affected by irradiation. Motivated by the work and the fact that TiO2 is stable in aqueous solutions we have used TiO2 films to coat the inner walls of microchannels and demonstrate changes in bulk EOF velocity upon exposure to UV irradation. Upon irradiating the electrolyte-electrode interface, electron-hole pairs are formed which can then reduce and oxidize species in the solution or adsorbed on the surface. Recombination of the electron-hole is a competing reaction. Based on the relative rates of the reactions, an excess or depletion of charge can be generated at the surface. Irradiation can modify the velocity profile, since the EOF velocity depends on the zeta potential that varies with the surface charge. The presence of electron or hole scavengers in the solution can influence the kinetics of the reactions and hence the surface charge and EOF velocity. The amount and type of charge accumulated at the surface also depends on the pH of the solution, type of ionic species and the ionic strength. Control of the bulk EOF velocity with light was demonstrated. Upon irradiation with UV light, negative charge accumulates on the TiO2 surface affecting the zeta potential and hence modulating the flow. The change in velocity with light depends on the pH of the solution. The largest change was observed at the point of zero zeta potential of TiO2 and glass. The modulation of surface charge with light on a TiO2 pattern will allow spatial and temporal control of the flow.

Integration of Functional Materials in Micro Fluidic Systems

The potential for the integration of functional materials with MEMS technologies to yield completely new approaches to fabrication is intriguing. By extending this line of reasoning to other functionality (conduction, surface energy, volume, modulus, electroluminesence, etc.) and the inclusion of multiple functionality within a single material one can envision a whole array of possibilities. On this project we developed the basic methodology for integrating responsive hydrogel polymer materials into microfluidic channels. In addition, we demonstrated valving and regulation functionality. The method utilizes liquid phase photopolymerization to fabrication components in situ (in side the channel) eliminating

assembly. These initial demonstrations lead to a larger DARPA project funded via the BioFLIPs program to develop completely integrated autonomous biosensing systems.

List of Publications Related to the Project

Journal Papers

Jensen, I., C. Johnson, A. Prakasam, R. Vijayendran and D. Leckband, "An Engineered Protein A Platform for Oriented, Immobilized Protein Arrays," submitted to *Bioconjugate Chemistry*, 2002.

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"Method of fabricating a microstructure," J. Bauer and D. Beebe, Provisional application #60/284,378, filed 4/17/01.

"Autonomous self-regulating microfluidic system," D. Beebe, J. Moore, R. Liu and D. Eddington, #P01314US, PAT filed 7/20/01.

"Microfabricated devices and method of manufacturing the same," D. Beebe and J. Moore, patent application #60/145,554 filed 9/24/99.

Collaborations Resulting From Project

CFD Research Corporation: As a result of the Project's funding, we were able to form a close collaboration with CFD Research Corporation. As part of this still ongoing collaboration, we have shared with CFDRC flow visualization and quantification data so that they can validate their code. These "test cases" include

- Bleached flow visualization data of bands in electrokinetic turns
- Bleached flow visualization experiments to measure diffusivity and electrophoretic mobility
- Electrokinetic injection data
- Velocity fields of 3000+ electrophoretic, Brownian particles in an electrokinetic flow
- Bulk fluid flow measurements for pressure and electrokinetic flows at an intersection of two channels

Coventor Corporation (formerly Microcosm): As in the case of our collaboration with CFDRC, we provided Coventor with electrokinetic flow visualization data that they used to validate their code. Specifically, we provided them with caged-fluorescence visualizations showing the effectiveness of the optimized turns we developed as part of this project.

Agilent Technologies: We worked closely with Agilent Technologies in our development of optimized microchannel turns for electrokinetics. Also, the electrokinetic micromixing work we initiated as part of this project (see Conference papers below) helped us initiate a close collaboration with Agilent Technologies in mixing and binding assay work. As a result of this collaboration, we have received gift funding from Agilent for our work in the area of micromixing.

Endovasix: The particle tracking velocimetry system we developed as part of this project has attracted much attention throughout the world. We have received inquiries for the details behind our particle tracking methods from Samsung (SAIT), Agilent, Sandia National Labs, Lawrence Livermore National Labs, and five universities in the U.S. and Japan. One example

where we have leveraged our unique expertise in particle tracking is a project for Endovasix corporation where we visualized the flow around 100 um laser-induced cavitation bubbles (forming and collapsing at rates in excess of 1 kHz). These experiments (performed in a blood like liquid) are unique and offer insight into fundamental cavitation phenomena in viscoelastic liquids.

Lawrence Livermore National Labs: The particle tracking and electrokinetics expertise we built during the life of this project was used to initiate a close collaboration with Lawrence Livermore Natl. Labs. Currently, we have two ongoing collaborations with LLNL: one on quantifying the effects of sonication on anthrax-like spores and one on quantifying the motion of non-spherical Brownian particles. LLNL is currently funding two students in my group.

ACLARA Biosciences: During this project (and subsequently), we received over a dozen microchannel systems which were gifted to us by ACLARA Biosciences. We used these channels in demonstrating and validating our particle tracking techniques and electrokinetic injection methods. In return, we have offered ACLARA key insight into the uniformity of the zeta potentials in their microchannels.

Staff & Students Supported

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- Apoorva Agarwal, M.S.candidate
- Joe Bauer, PhD, Theoretical and Applied Mechanics, UIUC
- G. Block, Ph.D. candidate
- Chuan-Hua Chen, M.S. Mechanical Engineering, Stanford University
- Shankar Devasenathipathy, M.S. Mechanical Engineering, Arizona State University
- Dave Eddington, MS, Biomedical Engineering, UW-Madison
- Edward Eteshola, Post-doc, Chemical Engineering, University of Illinois (now at the Cleveland Clinic)
- Rico Gunawan, University of Illinois
- Amy Herr, Ph.D. Mechanical Engineering, Stanford University
- Nickolay Lavrik, Post-doc Chemical Engineering, University of Illinois (now at University of Tennessee)
- Robin Liu, PhD, Mechanical Engineering, UIUC
- Jim Mikkelson, Ph.D. Chemistry, Brown University (now Consulting Professor in Chemical Engineering)
- Joshua I. Molho, Ph.D. Mechanical Engineering, Stanford University (now at Caliper Technologies)
- Bruce Mosier, Eng. Degree, Mechanical Engineering, Stanford University (now at Sandia National Labs)
- Kathy Motesgood, Technician, UIUC
- Mike Olsen, Post-doc, Theoretical and Applied Mechanics, UIUC
- Juan Santiago, post –doc, UIUC
- Kendra V. Sharp, PhD, Theoretical and Applied Mechanics, UIUC

- Fernando Siso-Nadal, obtained an MS while partially supported by the project; continued PhD studies at Cambridge University, UK, obtaining the PhD in 2002.
- Mark A. Stremler, Post-doc, Theoretical and Applied Mechanics, UIUC
- Derek Tretheway, University of California Santa Barbara
- Ravi Vijayendran, MS Chemical Engineering, University of Illinois (now at Caliper)
- Dazhi Wang, PhD Student, University of California Santa Barbara
- Steven T. Wereley, University of California Santa Barbara
- E. Yamaguchi, Ph.D. candidate
- Qing Yu, PhD, Chemistry, UIUC
- Bin Zhao, Post-doc, Chemistry, UIUC